Temperature/pH-Sensitive Hydrogels Prepared from Pluronic Copolymers End-Capped with Carboxylic Acid Groups via an Oligolactide Spacer

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The Pluronic F127 triblock copolymer was end-capped by carboxyl groups using a degradable oligolactide as a spacer to confer pH- and thermo-sensitive properties. With increasing chain length of the oligolactide, the temperature-dependent sol-gel transition curve was significantly shifted to higher concentration with concomitant narrowing of the gelation temperature range. Carboxylic acid end-capped Pluronic also exhibited a peculiar pH-dependent sol-gel transition behavior. At 37 °C, sharp gel-to-sol and sol-to-gel transitions were observed around pH = 4.8 and 8.2, respectively. The pH-dependent phase transition was caused by introduction of carboxylic acid groups at the ends of Pluronic F127.

Introduction

Stimuli-sensitive polymers that display physicochemical responses to slight changes of their environments have attracted considerable attention.[1–3] Among them, temperature/pH-sensitive polymers exhibiting a sol-gel transition under physiological conditions are useful for diverse biomedical applications such as delivery of bioactive macromolecules, tissue engineering, cell manipulation, and bioseparation.[4–7] Various synthetic polymers such as poly(N-isopropylacrylamide),[8,9] poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide) Pluronic triblock copolymers,[10,13] and poly[(lactic acid)-co-(glycolic acid)]-block-poly(ethylene oxide)-block-poly[(lactic acid)-co-(glycolic acid)] triblock copolymers[12–14] have been shown to exhibit temperature-sensitive phase transition behaviors in aqueous solution. At high concentrations, they self-assemble to form a highly water-absorbing three-dimensional gel network in response to temperature.

Pluronic triblock copolymers exhibit a wide range of sol-gel transition behaviors in aqueous solution with...
varying gelation temperatures depending on the polymer composition and concentration.\cite{15,16} Pluronic copolymers are known to self-assoc is, a few hydrogel solutions, which are closely packed to produce a physically cross-linked hydrogel structure above a certain concentration and temperature.\cite{17–19} While the Pluronic copolymers exhibit temperature-sensitive properties, they do not show any pH-sensitive property due to the lack of ionizable groups in the structure.\cite{20}

In this study, Pluronic copolymers that exhibit dual sensitivities to temperature and pH were synthesized. A series of carboxylic acid end-capped Pluronic copolymers were prepared by using oligolactide with different chain lengths as a degradable spacer. Pluronic F127 was used as an initiator for ring-opening polymerization of \( \text{D,LL} \)-lactide to produce oligolactide-Pluronic-oligolactide with a desired chain length, and carboxylic acid groups were introduced at the ends. Their temperature- and pH-dependent sol-gel transition behaviors were compared with unmodified Pluronic F127.

### Experimental Part

#### Synthesis of Oligolactide-\( \text{block} \)-Pluronic-\( \text{block} \)-Oligolactide Copolymer End-Capped with Carboxylic Acids

Pluronic F127 [(PEO)$_{99}$(PPO)$_{69}$(PEO)$_{99}$, 1 mmol] and \( \text{D,LL} \)-lactide with various monomer/initiator ratios were added in a two-necked round-bottomed flask, purged with dry nitrogen, and then reacted in toluene (50 mL) at 130 °C for 12 h using stannous octoate (4 mmol) as a catalyst. The molar ratio of monomer/catalyst was kept at 100:1 in the polymerization reaction. The polymer was precipitated into excess of cold ethyl ether and the residual solvent was removed under vacuum. \( \text{HO} \)-[(\text{oligoDLLA})$_n$-\( \text{block} \)-Pluronic-\( \text{block} \)-(\text{oligoDLLA})$_n$]-\( \text{OH} \) (1 mmol, \( n = 0, 8, \) and 18), succinic anhydride (SA, 4 mmol), and 4-(dimethylamino)pyridine (DMAP, 4 mmol) were dissolved in 1,4-dioxane (70 mL) and reacted in nitrogen atmosphere for 24 h at room temperature. The product was obtained by precipitation in hexane and evaporation under vacuum for 3 d. The degree of polymerization and the extent of substitution were determined by $^1$H NMR (Bruker AVANCE 400 spectrometer operating at 400 MHz) in chloroform-\( d_3 \). The terminal $^1$H protons produced by reaction with SA were confirmed at 2.62 ppm in the $^1$H NMR spectrum of the polymer.\cite{21} The degree of substitution was almost 100%.

$^1$H NMR (CDCl$_3$): $\delta = 5.21$–5.14 (br, \( \text{CH} \) of lactide), 3.88–3.06 (br, \( \text{CH}_2\text{CH}_2 \) of PPO and PEO), 2.62 (t, \( \text{CH}_2\text{CH}_2 \) of succinyl group), 1.51–1.46 (br, \( \text{CH}_3 \) of lactide), 1.16–1.05 (br, \( \text{CH}_3 \) of PPO).

#### Sol-Gel Transition Phase Diagram

The sol-gel phase transition temperatures for \( \text{HOOC} \)-[(\text{oligoDLLA})$_n$-\( \text{block} \)-Pluronic-\( \text{block} \)-(\text{oligoDLLA})$_n$]-\( \text{COOH} \) (\( n = 0, 8, \) and 18) were determined in pH = 7.4 phosphate-buffered saline (PBS) solution using a test tube inverting method with a 2-mL test tube and temperature increment of 1 °C. Each sample with a given concentration was added to the PBS solution for 12 h at 4 °C and heated gradually. A gel state was determined by inverting the vial when no fluidity was visually observed for 1 min. The sol-gel phase diagram determined by this method is known to have a precision of ± 0.5 °C.

### Rheological Studies

Dynamic modulus values of hydrogel samples at different pH values were measured by advanced rheometric extended systems (ARES, The Rheometric Science Inc., NJ) with a cone and plate (20 mm diameter plate, 4° cone angle) fixture. The gap between the cone and the plate was adjusted to 0.05 mm and the measurement was performed through a dynamic frequency sweep test in the range of 0.1–100 rad·s$^{-1}$ with a constant strain (10%) in a temperature controlled environment within 37 ± 0.2 °C. Storage (\( G' \)) and loss (\( G'' \)) moduli of each sample were measured as a function of frequency (\( \omega \)), and tan \( \delta = G''/G' \) was calculated and plotted.

### Results and Discussion

HO-[\( \text{oligoDLLA} \) \( _n \) -\( \text{block} \)-Pluronic-\( \text{block} \)-(\text{oligoDLLA})$_n$]-\( \text{OH} \) (\( n = 0, 8, \) and 18) was first prepared by the ring-opening polymerization of \( \text{D,LL} \)-lactide with Pluronic with \( \text{Sn}(\text{Oct})_2 \) as a catalyst, and its two terminal hydroxyl groups were converted to carboxylic acid groups to produce \( \text{COOH} \)-[(\text{oligoDLLA})$_n$-\( \text{block} \)-Pluronic-\( \text{block} \)-(\text{oligoDLLA})$_n$]-\( \text{COOH} \) (\( n = 0, 8, \) and 18) (Scheme 1). The number of lactic acid units in the oligolactide segment and the end group carboxyl functionalization were analyzed by $^1$H NMR spectroscopy.

The temperature-dependent sol-gel transition phase diagrams of carboxylic acid-terminated Pluronic derivatives at pH = 7.4 were significantly altered as shown in Figure 1. When carboxylic acid groups were introduced at the terminal ends of Pluronic F127, the sol-gel curve shifted to a higher concentration regime. With increase in the number of lactic acid units in the oligolactide spacer, the critical gelation concentration increased to a greater extent along with narrowing the gelation temperature range. It is known that the Pluronic copolymers aggregate with increasing temperature in the form of spherical micelles by enhanced hydrophobic interactions between PPO middle blocks of the copolymers. Above a critical concentration, the self-assembling Pluronic micelles are closely packed to generate a physically cross-linked hydrogel structure.\cite{22–24} The additional presence of ionic carboxylic acid group moiety at the PEO distal end of Pluronic F127 might disturb the well-ordered micellar packing structure due to the ionic repulsion between anionic micelles, requiring higher critical gel concentration to maintain a gel state. The incorporation of an oligolactide spacer...
segment adjacent to the carboxylic acid group might additionally lead to a much less ordered micellar packing structure in the gel formation, resulting in the shift of critical gel concentration to the higher concentration region. It can be seen that increasing the lactic acid monomer unit in the oligolactide spacer adjacent to Pluronic copolymer tends to increase the gelation temperature. This was probably caused by partitioning a relatively hydrophobic oligolactide segment into the PPO core, disturbing the micellar integrity as well as packing density, thereby raising the gelation temperature. Overall, a small variation in hydrophilic/hydrophobic balance of Pluronic F127 seems to greatly influence the micellar structure, integrity, and packing, resulting in a significant change in the sol-gel transition phase diagram.

Figure 2(A) shows the pH-dependent sol-gel phase diagram of HOOC–[(oligo-DLLA)$_n$-block-Pluronic-block-(oligo-DLLA)$_n$]–COOH ($n = 0, 8, \text{ and } 18$). Polymer concentrations for the lactic acid unit of 0, 8, and 18 polymers
were adjusted at 18, 23.6, and 29.1 wt.-%, respectively. These concentrations are near the critical gelation concentration values determined from the phase diagram curves in Figure 1. pH-sensitive sol-gel transition behavior below pH = 4 and above pH = 10 could not be obtained because oligolactide chains were very unstable under such conditions probably due to the hydrolytic scission of the oligolactide spacer. Carboxylic acid terminated Pluronic oligolactide spacer.

Carboxylic acid terminated Pluronic F127 shows a broad pH-dependent gel-sol-gel transition curve at 37 °C, while the introduction of oligolactide sequence in the carboxylated Pluronic copolymers exhibits very sharp transitions: gel-to-sol transition near pH = 5 and sol-to-gel transition near pH = 8 at a constant temperature. The gel-to-sol transition at pH = 5 occurs due to deprotonation of the terminal carboxylate group (−COO−) at its pK_a value (pK_a = 4.0). The deprotonated carboxylate groups in the periphery of Pluronic micelles are likely to hold water molecules more tightly, preventing the dense micellar packing from gelation. Interestingly, the sol-to-gel transition behavior was also observed at pH above 8. This peculiar gelation effect at pH above 8 can be explained by the decreased solubility of PEO chain in aqueous solution under basic pH conditions. It was previously shown that the solubility of hydrophilic PEO chain decreases with increasing pH, especially above pH 8. This peculiar gelation effect at pH above 8 can be due to deprotonation of the terminal carboxylate group (−COO−) at its pK_a value (pK_a = 4.0). The deprotonated carboxylate groups on the distal end of PEO chains under acidic conditions and decreased solubility of PEO chain under basic conditions were dually responsible for the pH-sensitive transition behaviors.

Thus, at the higher pH condition, the reduced PEO solubility made the PEO shell layer dehydrated, leading to the formation of more compact micellar packing even though terminal carboxylic acid groups were ionized. This would lead to the gel-to-sol transition observed at pH = 8.

Schematic pH-dependent gelation behavior for carboxylated oligolactide Pluronic F127 is illustrated in Figure 2(B). Rheological properties at different pH values were determined as shown in Figure 3. Viscoelastic loss tangent (tan δ = G'/G’) values of HOOC−[(oligo-DLLA)_8-block-Pluronic-block-(oligo-DLLA)_8]−COOH (23.6 wt.-%) were measured at 37 °C by an oscillatory shear rheometer under various pH conditions (pH = 4, 6, 7, 7.4, and 10). The value of tan δ provides a relationship between viscous and elastic natures of polymer solutions. The tan δ values for the carboxylic acid end-capped Pluronic solution at pH 6, 7, and 7.4 were larger than 1, indicating that the solution was in a sol state. However, the values became less than 1 at pH = 4 and 10, suggesting that the polymer solution transformed into a soft viscoelastic solid state. The pH-dependent rheological properties are consistent with the pH-dependent sol-gel transition behaviors as shown in Figure 2. The above results directly support that carboxylic acid terminated Pluronic-oligolactide copolymers have pH-sensitive transition behaviors.

Conclusion

Temperature-/pH-sensitive Pluronic copolymers were prepared by end-capping carboxyl groups at the terminal groups of PEO. The pH-sensitive transition behaviors reveal how well spherical Pluronic micelles were clustered and packed together for the gel formation. It appears that the protonation/deprotonation degree of carboxylic acid groups on the distal end of PEO chains under acidic condition and decreased solubility of PEO chain under basic condition were dually responsible for the pH-sensitive gel-sol-gel transition over a broad range of pH values.

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Figure 3. Rheological behavior of HOOC−[(oligo-DLLA)_8-block-Pluronic-block-(oligo-DLLA)_8]−COOH at different pH values.